

## Immunofluorescent on histological sections

Prepare sections from blocks embedded in OCT using the 2-methylbutane method (see cryoprotection and processing of embryonic tissue protocol)

For paraffin sections, dewax and rehydrate sections

### Day1:

1. Rinse in PBS for 5min at RT.
2. Antigen retrieval (this is only necessary for paraffin sections or for nuclear proteins):
  - a) Prewarm 10mM citric acid pH6.0 for 20 min in a steamer.
  - b) Put slides in the prewarmed citric acid for 20 min in the steamer.
  - c) Remove jar from the steamer and cool it at RT for about 40min.
3. Fix in 4% PFA (paraformaldehyde)/PBS for 5min at RT.
4. Rinse in PBS twice for 5min at RT.
5. Hydrogen peroxide (0.2% in PBS) for 20min at RT (optional).
6. 0.5% TritonX-100\* in PBS for 30 min at RT.
7. Rinse 2x in PBT (0.2% tritonX-100/PBS) for 5min each at RT.
8. Block in 10% donkey serum/PBT for 2h at RT in a humid box.
9. Prepare primary Ab in 1% donkey serum\* in PBT.
10. Add 220-300 $\mu$ l of Ab solution per slide and incubate in a humidified box at 4°C overnight. (you can gently put a parafilm strip (slightly smaller than the slide to prevent evaporation).

### Day2:

11. Allow sections to come to RT (about 5 min).
12. Wash with PBT 5 times for 5 min each at RT.
13. Prepare secondary Ab (1:500 dilution in 1% donkey serum/PBT).
14. Add 300 $\mu$ l secondary Ab solution per slide and incubate for 2 hours at RT in a dark, moist chamber
15. Wash with PBT five times, 10min each at RT in Coplin jar (covered in foil)
16. Counter stain with Hoechst 33342 from molecular probes (H-3570; 5 $\mu$ l/50ml PBT) for 5min at RT in a Coplin jar covered in foil. (Hoechst solution can be recycled and stored at 4°C).
17. Rinse with PBS twice.
18. Coverslip with Biomedex Gel/Mount (M01) in dark.

\* Different concentration of detergent for different types of antigens (0.5% for nuclear proteins; 0.3% for cytoplasmic proteins; no detergent for membrane proteins.

\*\* Make sure that the blocking serum and the primary antibody are derived from different species, eg. donkey serum **should not be used** if the primary ab is also from donkey. Otherwise you will have a very high background.